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Interferon-γ mediated up-regulation of caspase-8 sensitises medulloblastoma cells to radio- and chemotherapy

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ABSTRACT

Loss of caspase-8 expression – which has been demonstrated in a subset of Medulloblastoma (MB) – might block important apoptotic signalling pathways and therefore contribute to treatment resistance. In this study, IFN-γ mediated up-regulation of caspase-8 in human MB cells was found to result in chemosensitisation to cisplatin, doxorubicin and etoposide, and sensitisation to radiation. These effects were more prominent in D425 and D341 MB cells (low basal caspase-8 expression) when compared to DAOY MB cells (high basal caspase-8 expression). IFN-γ mediated chemosensitisation and radiosensitisation effects were reduced by treatment with the caspase-8 specific inhibitor z-IETD-fmk. Treatment of IFN-γ resulted in activation of STAT1 in DAOY MB cells and to a lesser extent in D425, but not in D341, indicating that IFN-γ acts in MB cells through STAT1-dependent and -independent signalling pathways. Taken together, our results demonstrate that IFN-γ mediated restoration of caspase-8 in MB cells might enhance apoptotic pathways relevant to the response to chemo- and radiotherapy.

1. Introduction

Medulloblastomas (MB) are the most common malignant central nervous system primary tumours in childhood and constitute more than 20% of all Paediatric brain tumours.[1] Most metastatic and recurrent childhood MB are resistant to current therapeutic approaches, including radiation and high-dose chemotherapy.[2–4] The success of anti-cancer therapies is often hampered by resistance to apoptosis, which may depend on defects in common apoptotic pathways.[5] Thus, characterising these alterations and identifying ways for the restoration of these pathways are a prerequisite for developing novel anti-tumour strategies.[6]

Caspase-8 expression, a core determinant of sensitivity to cell death, is frequently impaired in resistant tumour cells and restoration of caspase-8 sensitised for drug-induced apoptosis.[7–9] Our own studies demonstrate that subsets of MB do not express the key initiator caspase-8 and that loss of caspase-8 is associated with an unfavourable survival outcome.[10,11] Interferon-γ or 5-aza-2′deoxycytidine mediated restoration of caspase-8 expression resulted in restoration of sensitivity to TRAIL-induced apoptosis.[11,12] These results are consistent with data published by others who demonstrated IFN-γ mediated up-regulation of caspase-8 in cancer cell lines derived from neuroblastoma,[8] Ewing's sarcoma,[13] colon carcinoma,[14] breast carcinoma[15] and cholangiocarcinoma.[16]

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IFN-γ is a pleiotropic cytokine that is involved in antiviral responses, immune surveillance, inhibiting cellular proliferation and tumour suppression.[17–19] IFN-γ exerts its effects on cells by interacting with a specific receptor composed of two subunits, IFN-γ receptor 1 (IFN-γR1) and IFN-γ receptor 2 (IFN-γR2).[18,19] Binding of IFN-γ to its receptor induces receptor oligomerisation and activation of the receptor-associated Janus kinases (Jaks) by trans-phosphorylation. The activated Jaks phosphorylate the intracellular domain of the receptor, which serves as a docking site for STAT1. STAT1 is then phosphorylated, dimerises and translocates to the nucleus and regulates gene expression by binding to IFN-γ-activated sequence elements in the promoters of IFN-γ-regulated genes.[20] This paradigm, which established a solitary role for STAT1 signalling pathways in promoting the cellular responses induced by IFN-γ, has been challenged recently by several studies.[21,22] These studies have experimentally proven that the IFN-γ receptor may regulate gene expression also by STAT1-independent pathways. Microarray analysis showed that a number of genes are regulated by IFN-γ in STAT1-null primary bone marrow-derived macrophages or mouse embryo fibroblasts.

In the current study, we investigated the effects of IFN-γ mediated caspase-8 restoration on chemo- and radiosensitivity of MB cells, and evaluated IFN-γ receptor expression in primary MB. Moreover, we investigated whether IFN-γ signalling in MB is STAT1-dependent or -independent.

2. Patients and methods

2.1. Primary tumour and normal brain samples

Frozen tumour tissue to perform real-time-polymerase chain reaction (RT-PCR) was available from 52 MB patients treated at the Children's Hospital of Philadelphia, PA ($n = 33$), the University Children's Hospital of Vienna, Austria ($n = 13$), and the University Children's Hospital of Zurich, Switzerland ($n = 6$). Tumour samples were the kind gifts of Dr. Peter Phillips (The Children's Hospital of Philadelphia, PA) and Dr. Irene Slavc (University Children's Hospital of Vienna, Austria). All diagnoses were confirmed by histological assessment by experienced neuropathologists of a tumour specimen obtained at surgery. Tumour samples were snap frozen in liquid nitrogen in the operating room and then stored at −80 °C until further analysis. The median age at diagnosis for these MB patients was 6.5 years (range: 0.3–32.3 years). Thirty-four (65%) were male and 18 (35%) were female. RNA of normal brain samples was purchased from Stratagene (La Jolla, CA) and Clonetech (Palo Alto, CA); they included foetal brain samples (18 and 19 weeks of gestation), cerebellum, frontal cortex (50 years of age), and whole brain (15 years of age). Near-normal brain samples were the kind gift of Dr. Peter Phillips (The Children's Hospital of Philadelphia, PA). They included cerebellum of a 4-year-old glioma patient, temporal cortex from a 4-year-old epilepsy surgery patient, temporal cortex from a 14-year-old epilepsy surgery patient and occipital cortex from a 19-year-old epilepsy surgery patient. Normal, non-commercial brain samples were snap frozen in liquid nitrogen in the operating room and then stored at −80 °C until further analysis.

2.2. Human MB cell lines

DAOY human MB cells were purchased from the American Type Culture Collection (Rockville, MD). D341, D425 and D458 human MB cells were the kind gift of Dr. Henry Friedman, Duke University, Durham, NC, USA. UW228-2 human MB cells were a kind gift from Dr. John R Silber, University of Washington, Seattle, WA, USA. CHOP707 human MB cells were a kind gift from Dr David Pleasure, The Children's Hospital of Philadelphia, PA, USA. DAOY, D341, D425 and D458 cells were cultured in Richter's Zinc Option medium/10% foetal bovine serum (1% non-essential amino acids was added to the medium of D341 and D425 cells). CHOP707 cells were cultured in RPMI/10% foetal bovine serum, and UW228-2 cells in DMEM/10% foetal bovine serum. All cell cultures were low (20–30) passages. They were maintained at 37 °C in a humidified atmosphere with 5% CO_2.

2.3. Quantitative RT-PCR

Isolation of total RNA and cDNA synthesis was performed as previously described.[10,23] Real-time PCR quantification of IFN-γR1 and IFN-γR2 mRNA was performed using Assays-on-Demand Gene Expression Products™ (Applied Biosystems; Rotkreuz, Switzerland) which consist of a mix of unlabelled PCR primers for IFN-γR1 or IFN-γR2 and TaqMan MGB probe (FAM dye-labelled). Primers and probes for the endogenous control 18S rRNA were purchased from PE Biosystems (Balgach, Switzerland). For each PCR run, a mix was prepared of either 1× Assays-on-Demand for IFN-γR1 or -R2 mix or 200 nM of each 18S rRNA primer, 400 nM of 18S rRNA probe, 100 ng of cDNA and 1× of PCR master mix (Applied Biosystems) in a final volume of 25 μl. The thermal cycling conditions consisted of an initial denaturation step at 95 °C for 10 min and a 50-cycle countdown at 95 °C for 15 s and 60 °C for 1 min. Experiments were performed in triplicate for each data point. Each sample was normalised on the basis of its 18S rRNA content. Relative expression of IFN-γR1 and IFN-γ R2 mRNA was calculated using the comparative threshold cycle (C_T) method described previously.[24] The amount of IFN-γR1 and IFN-γ R2 mRNA normalised to 18S rRNA was related to the commercially available calibrator human cerebellum (BD Clontech, Basel, Switzerland).

2.4. Caspase-8 activity

Caspase-8 activity was measured by using a caspase-8 assay kit according to the manufacturer's instructions (Calbiochem; Lucerne, Switzerland). Based on results from our previous work,[11] DAOY, D341 and D425 human MB cells were treated with different concentrations of IFN-γ (10 or 100 U/ml) for 48 h in the presence or absence of the caspase-8 specific inhibitor z-IETD-fmk (50 μM). Equal amounts of protein from cell extracts were used for each sample. Cell lysates were transferred into 96-well plates, and 50 μl assay buffer was added to each well followed by 10 μl caspase-8 substrate conjugate. The plates were incubated at 37 °C for 2 h and then read with a fluorescent plate reader measuring excitation at 400 nm and emission at 505 nm.

2.5. Chemo-sensitivity assay

Cell viability of human MB cells DAOY, D341 and D425 were quantified using a colorimetric 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulphophenyl)-2H-tetrazolium inner salt (MTS) assay (Promega; Wallisellen, Switzerland) as previously described.[12,25] Briefly, 100 μl of target cell suspension containing 3–4 × 10^3 MB cells was added to each well of 96-well microtitre plates, and each plate was incubated at 37 °C in a humidified 5% CO_2 atmosphere. Following 48 h of 100 U/ml IFN-γ (Roche Diagnostics; Basel, Switzerland; solved in PBS) pre-incubation in the presence or absence of the caspase-8 inhibitor z-IETD-fmk (Calbiochem; Lucerne, Switzerland; reapplied every 48 h without removal of IFN-γ), cells were treated for 72 h with various concentrations of chemotherapeutic drugs, as indicated. Ten microlitres of MTS working solution were then added to each culture well and the cultures were incubated for 1–4 h at 37 °C in a humidified 5% CO_2 atmosphere. Each condition was performed in triplicate. The absorbance values of each well were measured with a microplate spectrophotometer (Molecular Devices, Sunnyvale, CA) at 490 nm. Results are presented as the mean percentage of survival ±SD ($n = 3$) compared to IFN-γ (100 U/ml) pre-treated or IFN-γ untreated control cells.

2.6. Radio-sensitivity assay

Exponentially growing DAOY, D341 and D425 human MB cells were seeded in 96-well plates. Following 48 h of 100 U/ml IFN-γ pre-incubation in the presence or absence of the caspase-8 inhibitor z-IETD-fmk (reapplied every 48 h without removal of IFN-γ), MB cells were irradiated with 2 Gy, 5 Gy or 10 Gy, using a Pantak Therapax 300 kV X-ray unit at 0.7 Gy/min. Dosimetry was controlled with a Vigilant dosimeter. Cell viability of human MB cells was quantified using the MTS assay 72 h after irradiation. Results are presented as mean percentage of survival ±SD ($n = 3$) compared to IFN-γ (100 U/ml) pre-treated or IFN-γ untreated control cells.

2.7. STAT1 activity assay

Nuclear protein extracts were obtained from MB cells (DAOY, D341 and D425; treated with 0 or 100 U/ml IFN-γ) by using the BD™ TransFactor Extraction Kit (BD Clontech; Basel, Switzerland) according to the manufacturer's instructions. Aliquots of nuclear protein were stored at −80 °C. The activation of STAT1 was measured using the Mercury TransFactor assay (BD Clontech), an enzyme-linked immunosorbent assay (ELISA)-based assay, according to the manufacturer's instructions. Briefly, 5 μg of nuclear protein samples were incubated for 1 h in a 96-well plate coated with an oligonucleotide that code for the STAT1 consensus binding site sequence and to which phosphorylated STAT1 contained in nuclear extracts specifically binds. After washing, antibody directed against STAT1 DNA complex (1:500 dilutions) was added to these wells and incubated for 1 h. Following incubation for 1 h with a secondary horseradish peroxidase-conjugated antibody (1:1000 dilution), specific binding was detected by colorimetric estimation at 650 nm. Using either mutant DNA or no protein addition controlled for non-specific binding. Furthermore, binding specificity between DNA and STAT1 protein was controlled for by using a competitor oligo having the same DNA sequence as the oligo of the coated wells.

2.8. Statistical analysis

Differences between means were examined for statistical significance with Student's *t*-test. $P < 0.05$ was considered to be significant. GraphPad Prism 4 (GraphPad Software, San Diego, CA, USA) software was used to calculate IC_{50} concentrations. IFN-γR1 and IFN-γR2 mRNA expressions between primary MB samples/MB cell lines and normal human brain samples were compared using the Mann–Whitney test.

3. Results

3.1. IFN-γ treatment sensitises MB cells to chemotherapy

To test whether IFN-γ alters sensitivity to chemotherapeutic drugs in MB cells, we selected human MB cells with low basal caspase-8 expression (D341 and D425) and DAOY MB cells with high basal caspase-8 expression.[12] We have previously shown that treatment with IFN-γ (10 or 100 U/ml for 48 h) results in restoration of caspase-8 mRNA and protein expression in D341 and D425 MB cells, but has no major effect on caspase-8 expression in DAOY MB cells.[11] In the current study, we show that IFN-γ mediated restoration of caspase-8 expression in D341 and D425 MB cells results in dose-dependent restoration of caspase-8 activity, which is blocked by the specific caspase-8 inhibitor z-IETD-fmk (50 μM) (Fig. 1). This is in contrast to DAOY MB cells, where high basal caspase-8 activity remains unaffected by IFN-γ treatment.

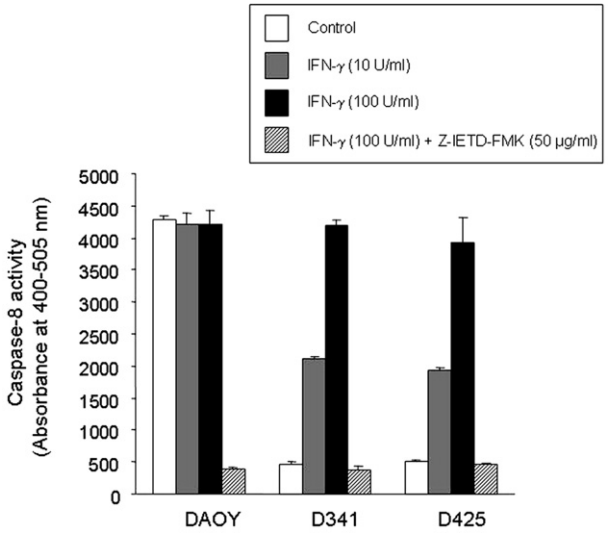

Fig. 1 – Treatment with IFN-γ (48 h) resulted in a dose-dependent significant increase of caspase-8 activity in D425 and D341 MB cells (low basal caspase-8 expression) but not in DAOY MB cells (high basal caspase-8 expression). In all three cell lines, caspase-8 activity was significantly reduced in the presence of the specific caspase-8 inhibitor z-IETD-fmk. Values represent the relative activity signal after subtraction of the buffer controls signal + SD ($n = 3$).

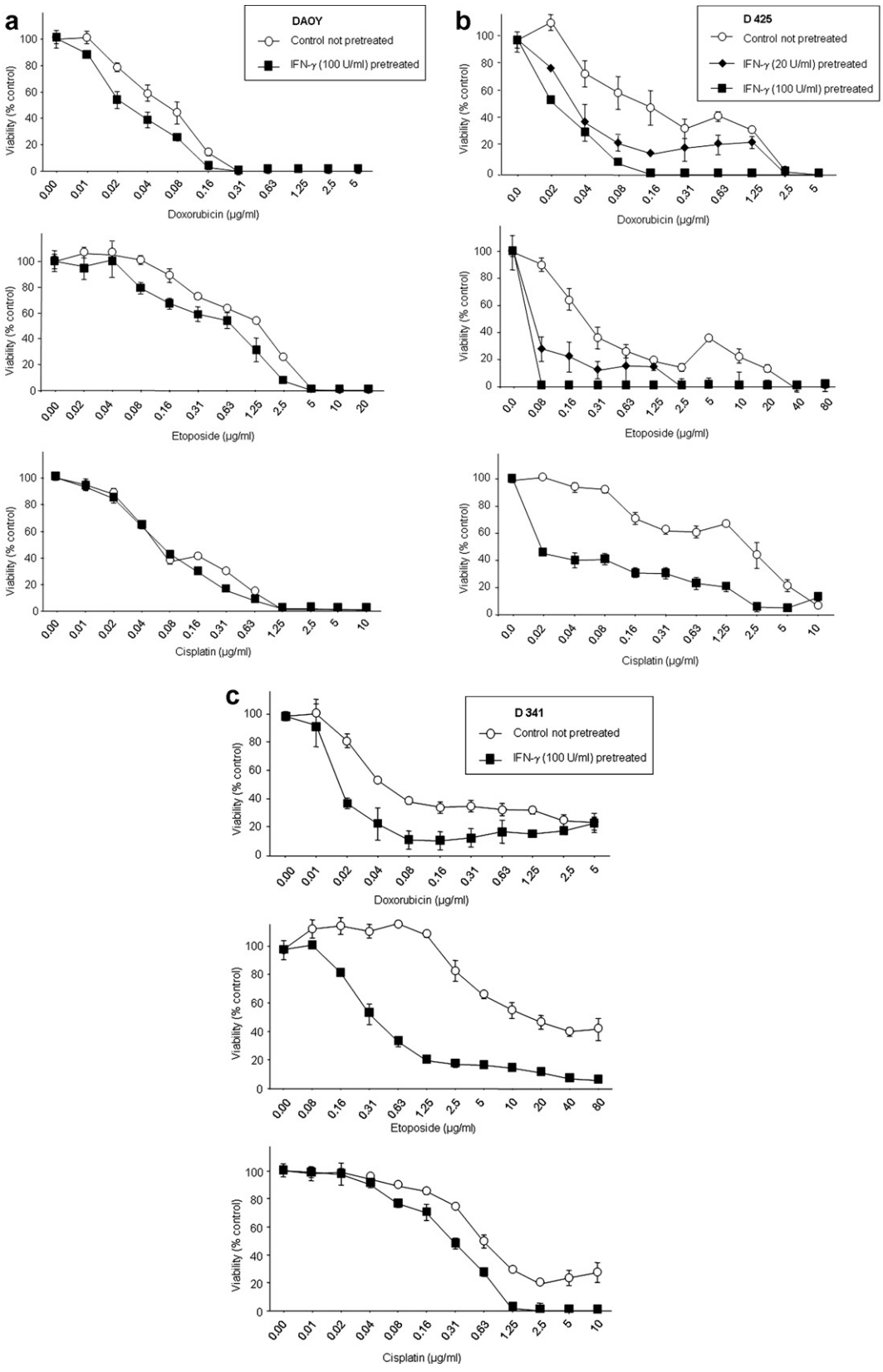

Fig. 2 – Pre-treatment with IFN-γ (48 h) resulted in significantly increased chemosensitivity of MB cells to doxorubicin (D425 > D341 > DAOY), etoposide (D425 > D341 > DAOY) and cisplatin (D425 > D341). These effects were more prominent in D425 and D341 MB cells (low caspase-8 expression) when compared to DAOY MB cells (high basal caspase-8 expression). Values represent the mean percentage of viability measured after 72 h incubation with chemotherapeutic drugs ±SD ($n = 3$).

After pre-treatment with IFN-γ during 48 h, the human MB cell lines DAOY, D341 and D425 were incubated with various concentrations of the cytotoxic drugs doxorubicin, etoposide and cisplatin for 72 h, and cell viability was assessed using the MTS assay. The three chemotherapeutic drugs tested caused dose-dependent inhibition of proliferation in all MB cell lines tested (Fig. 2). Pre-treatment with IFN-γ (100 U/ml) resulted in significant increases of chemosensitivity of caspase-8 low basal level D341 (doxorubicin, IC50: 0.016 versus 0.030 μg/ml; etoposide, IC50: 0.308 versus 3.533 μg/ml; cisplatin, IC50: 0.298 versus 0.462 μg/ml) and D425 human MB cells (doxorubicin, IC50: 0.022 versus 0.100 μg/ml; etoposide, IC50: <0.080 versus 0.192 μg/ml; cisplatin, IC50: 0.017 versus 1.830 μg/ml), but only in minor differences in caspase-8 high basal level DAOY human MB cells (doxorubicin, IC50: 0.025 versus 0.055 μg/ml; etoposide, IC50: 0.570 versus 1.140 μg/ml; cisplatin, IC50: 0.059 versus 0.071 μg/ml).

3.2. *IFN-γ treatment sensitises MB cells to radiation*

After pre-treatment with IFN-γ during 48 h, the human MB cell lines DAOY, D341 and D425 were irradiated with 0, 2, 5 and 10 Gy and cell viability was assessed 72 h later using the MTS assay. Irradiation caused dose-dependent inhibition of proliferation in all MB cell lines tested (Fig. 3). Pre-treatment with IFN-γ resulted in significant increases of radiosensitivity of D341 and D425 human MB cells (low basal caspase-8 expression). In the DAOY human MB cells (high basal caspase-8 expression); the increase of IFN-γ mediated radiosensitivity was less pronounced, but still significant.

3.3. *Caspase-8 dependency of IFN-γ mediated sensitisation for chemo- and radiotherapy*

We then tested IFN-γ enhanced susceptibility of DAOY, D341 and D425 to doxorubicin, etoposide or ionising radiation in the presence of a specific inhibitor for caspase-8 (z-IETD-fmk, 50 μM). IFN-γ enhanced susceptibility of DAOY, D341 and D425 to chemotherapy was significantly reduced in the presence of z-IETD-fmk (Fig. 4). To a lesser extent, but still reproducible, IFN-γ enhanced susceptibility of MB cells to irradiation was also reduced (Fig. 5). Therefore, IFN-γ-mediated chemo- and radiosensitisation appears to be (at least partly) caspase-8 dependent.

3.4. *Cellular responses to IFN-γ in MB cells*

To study the cellular responses to IFN-γ in MB cells, the nuclear extracts of DAOY, D341 and D425 cells that were either untreated or treated with 100 U/ml IFN-γ were probed with a STAT1-specific DNA-binding domain sequence to which activated STAT1 binds competitively and were assayed for IFN-γ dependent STAT1 activity as explained in the material and methods section. Whilst treatment with IFN-γ led to clear STAT1 activation in DAOY cells, IFN-γ had only a minor effect on STAT1 in D425 cells. Of most interest was the observation that treatment with IFN-γ had no effect at all on STAT1 activity in D341 cells (Fig. 6). These results indicate that IFN-γ induces its effects on human MB cells through STAT1-dependent and -independent signals.

3.5. *Expression of IFN-γ receptors in MB cell lines and in primary MB compared to normal brain samples*

To study relevant components of the IFN-γ signalling pathway, we determined the mRNA expression of IFN-γR1 and -R2 in 6 MB cell lines, 52 primary MB and 13 normal brain samples using real-time RT-PCR (Fig. 7). In all MB cell lines tested, both IFN-γR1 and -R2 were expressed. For IFN-γR1, no significant differences were found between primary MB samples, MB cell lines and normal brain samples (Mann–Whitney U test, $p = 0.34$ and $p = 0.38$, respectively). However, IFN-γR2 mRNA expression levels in primary MB (median 1.45, range 0.06–4.42) were significantly lower than IFN-γR2 mRNA expression levels in normal brain samples (median: 3.15, range: 0.65–9.03; Mann–Whitney U test, $p < 0.05$). No significant correlations were found between IFN-γR1 and IFN-γR2 mRNA expression levels and age or gender (data not shown).

4. Discussion

Caspase-8 plays an essential role in apoptosis induced by activated death receptors, but might also be involved in apoptosis induced by chemotherapeutic agents and irradiation.[26–32] Drug resistance and/or resistance to irradiation has obvious selective advantages for tumour cells and might explain why patients whose MB express low levels of caspase-8 have

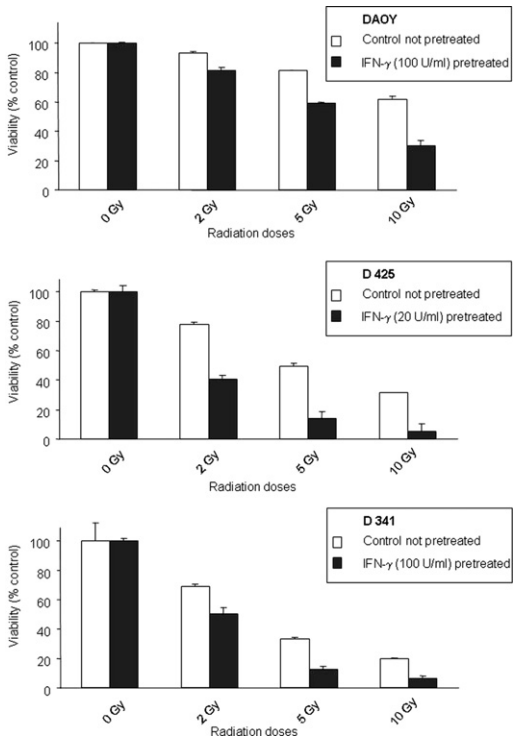

Fig. 3 – Pre-treatment with IFN-γ (48 h) resulted in significantly increased radiosensitivity of MB cells. These effects were more prominent in D425 and D341 MB cells (low caspase-8 expression) when compared to DAOY MB cells (high basal caspase-8 expression). Values represent the mean percentage of viability measured 72 h after irradiation ±SD ($n = 3$).

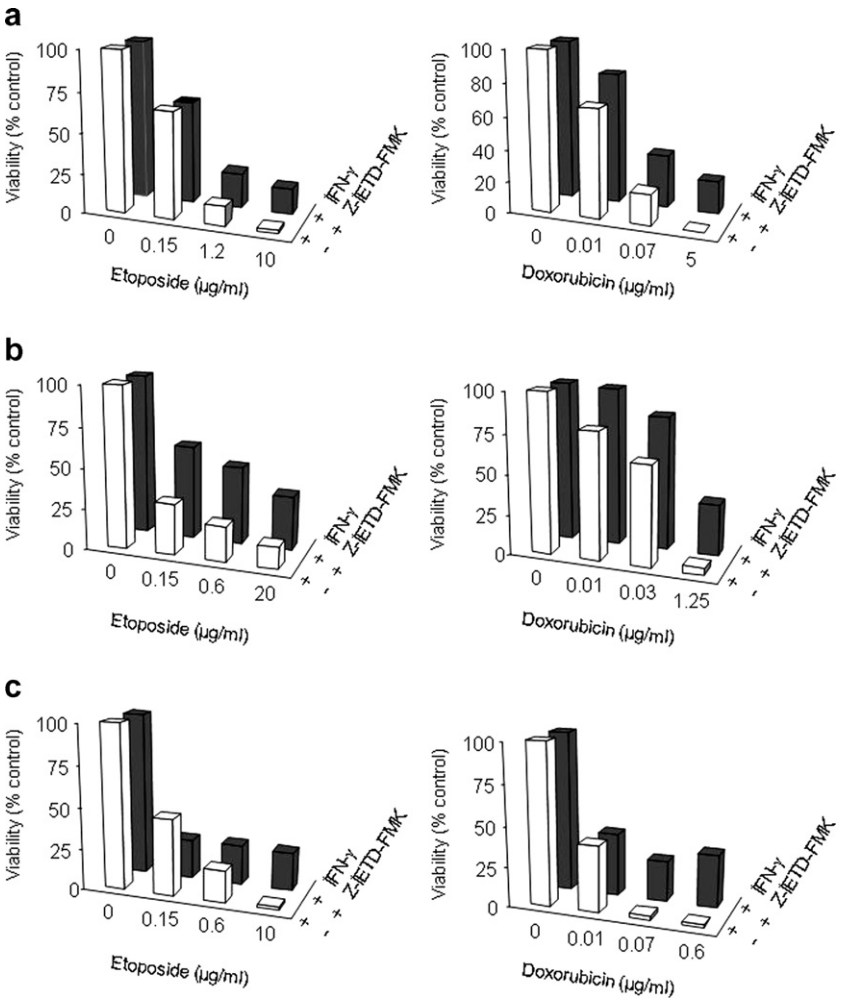

Fig. 4 – The chemo-sensitisation effects to doxorubicin and etoposide mediated by IFN-γ are reduced in the presence of the caspase-8 inhibitor z-IETD-fmk in the MB cell lines DAOY (a), D425 (b) and D341 (c). Values represent the mean percentage of viability measured after 72 h incubation with IFN-γ, cytotoxic drugs and z-IETD-fmk compared with z-IETD-fmk untreated cells ±SD (n = 3).

a less favourable prognosis than patients whose MB express higher levels of caspase-8.[11] Accordingly, restoration of caspase-8 might be of clinical benefit.

Up-regulation of caspase-8 through treatment with IFN-γ has been recently demonstrated in osteosarcoma,[33] breast cancer,[15] colon carcinomas,[34] and leukaemia cells.[35] In our own studies, we have demonstrated IFN-γ mediated dose-dependent restoration of caspase-8 mRNA and protein expression in the caspase-8-negative MB cell lines D425 and D341.[11] Here, we demonstrate that IFN-γ treatment not only restores caspase-8 expression but also activity.

We then investigated whether IFN-γ mediated up-regulation of caspase-8 might result in alteration of sensitivity to chemotherapy. We found that pre-treatment with IFN-γ resulted in sensitisation of MB cells to cisplatin (D425 and D341), doxorubicin (D425, D341 and DAOY) and etoposide (D425, D341 and DAOY). In D425 and D341 MB cells with low basal caspase-8 expression, this effect was more prominent than in DAOY MB cells expressing high levels of caspase-8. In addition, chemosensitisation was reduced by the caspase-8 specific inhibitor z-IETD-fmk. This suggests that IFN-γ-mediated enhancement of chemosensitivity is caspase-8 dependent. These results are consistent with recent findings from the literature where IFN-γ mediated facilitation to apoptosis in neuroblastoma cells was inhibited by the caspase-8 specific inhibitor z-IETD-fmk.[36]

Although radiation therapy dose reduction is being pursued for children with standard-risk MB, strategies for enhancing the efficacy of irradiation are currently examined in those with high-risk MB. These include hyperfractionated radiation delivery and radiosensitisation.[37] We therefore also studied radiosensitivity, and found that IFN-γ mediated up-regulation of caspase-8 resulted in sensitisation of MB cells to radiation. This effect was more prominent in D425 and D341 cells (low basal caspase-8 expression) when compared to DAOY MB cells (high basal caspase-8 expression). Radiosensitisation was reduced by the caspase-8 specific inhibitor z-IETD-fmk. These data are in accordance with previous results by others showing that caspase-8 is critically involved in enhancing radiation induced cytotoxicity[38] and IFN-γ enhanced radiation-induced cell death in breast cancer,[39] and leukaemia cells.[40]

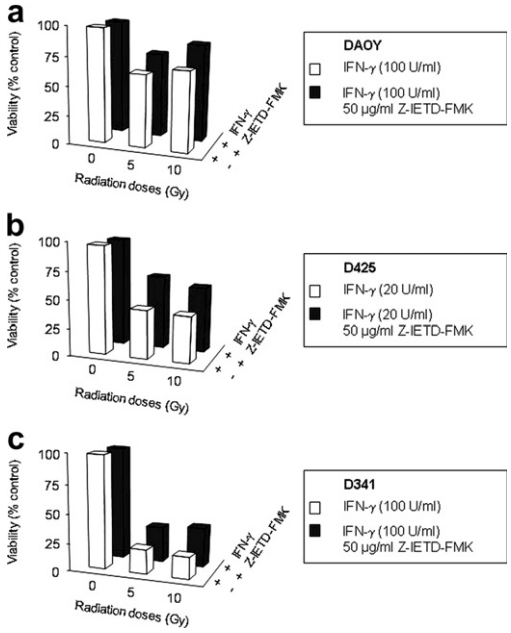

Fig. 5 – The radio-sensitisation effects, mediated by IFN-γ are blocked in the presence of the caspase-8 inhibitor z-IETD-fmk in the MB cell lines DAOY (A), D425 (B) and D341 (C). Values represent the mean percentage of viability measured after 72 h incubation with IFN-γ, z-IETD-fmk and irradiation compared with z-IETD-fmk untreated cells ±SD (n = 3).

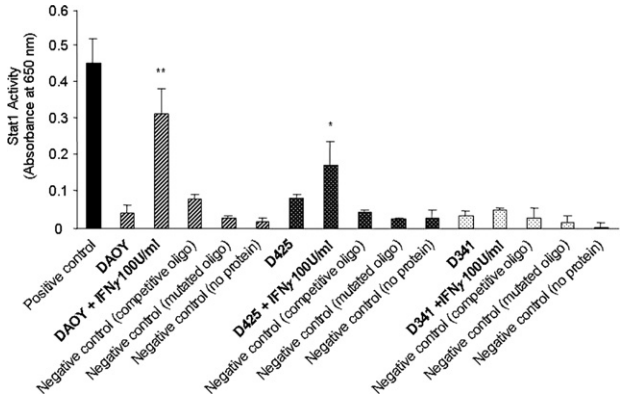

Fig. 6 – STAT1 activation upon treatment with IFN-γ determined by the Mercury TransFactor assay. Controls included nuclear extract of U-937 cell line treated with IFN-γ (positive control) and competitive oligonucleotides, oligonucleotides and no protein (negative control). Whilst STAT1 activity was clearly positive in DAOY, STAT1 activity was weak in D425 and negative in D341 human MB cells. Values represent the mean of OD at 650 nm compared with (no protein) negative controls ±SD (n = 3). **DAOY significantly different from control values, determined by Student's t-test ($p < 0.005$). *D341 significantly different from control values, determined by Student's t-test ($p < 0.05$).

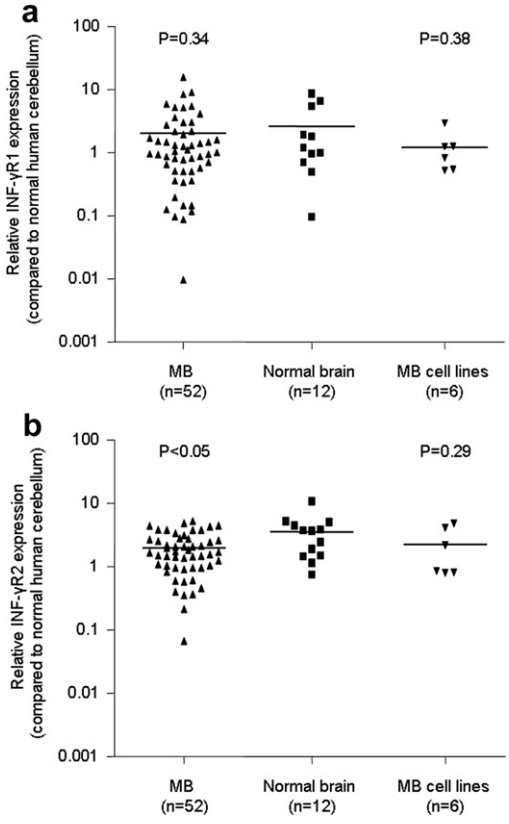

Fig. 7 – Human IFN-γR1 and IFN-γR2 mRNA expression in 52 primary MB, 13 normal brain samples, and in 6 MB cell lines was determined by quantitative real-time-polymerase chain reaction (RT-PCR) using 18 S rRNA as an endogenous control and normal human cerebellum as a calibrator. Compared with normal brain samples, IFN-γR2 mRNA expression levels were significantly lower in primary MB.

Although there is a previous report showing that up-regulation of caspase-8 in D283 Med MB cells by IFN-γ is mediated through the STAT1 pathway,[8] here, we demonstrate by analysing three other MB cell lines that IFN-γ signalling in MB is mediated through STAT1-dependent and -independent pathways. Our results are in accordance with a previous report[41] demonstrating that STAT1 might not be required for up-regulation of caspase-8 and with recent studies demonstrating that IFN-γ may also function independently of STAT1 to affect the growth of tumour cells.[21,22]FN-γ mediated STAT-1 independent signalling is thought to result from recruitment of signalling proteins that have SH2 domains to the phosphotyrosine residues of Jak kinases or IFN-receptor chains.[42] However, the identity and the substantial role of the STAT1 independent molecules which are supposed to be involved in INF-γ-mediated up-regulation of caspase-8 and sensitisation to chemotherapy in both D425 and D341 medulloblastoma cells are still elusive since the detailed pathways have not been established. Clearly, additional experiments including, e.g. a STAT1 dominant negative cell line would be of interest to decipher further the IFN-γ-mediated signalling in human MB cells.

IFN-γ is a pleiotropic cytokine secreted by activated T-lymphocytes and natural killer cells. IFN-γ is involved in antiviral responses, immune surveillance, inhibiting cellular proliferation and tumour suppression.[17,19] Several lines of evidence suggest that IFN-γ mediates an array of pathophysiological effects on the central nervous system.[43–45] Transgenic mice ectopically expressing IFN-γ in the CNS show hypomyelination and abnormal proliferation, differentiation

and migration of cerebellar granule neurons.[46,47] More recently, Lin et al.[48] demonstrated in a mouse model that IFN-γ expression in a very narrow developmental window of the perinatal period can induce sonic hedgehog expression and medulloblastoma in the cerebellum. Interestingly, once the tumours were established, continued expression of IFN-γ seemed to induce host responses with extensive macrophage and lymphocytic infiltration and tumour necrosis and apoptosis.

Several observations suggest that signals resulting from the binding of IFN-γ to its receptor depend on the number of surface receptors transducing the IFN-γ signal.[49] By demonstrating that IFN-γ receptors knockout mice developed tumours more rapidly and frequently than did the wild-type controls, Kaplan et al. revealed the action of IFN-γ on the tumour cells as an extrinsic tumour-suppressor mechanism.[50] Accordingly, cancer cells might avoid the effect of IFN-γ by either reducing the expression of IFN-γ receptors or blocking them.

In this study, we studied the IFN-γ receptor components on the mRNA levels and we made two observations. First, all MB cell lines and primary MB samples tested express mRNA of the cellular receptors for IFN-γ. Second, the mRNA expression of IFN-γR2 was significantly lower in primary MB when compared to normal brain samples. Therefore, reduced IFN-γ signalling might be involved in MB biology.

Taken together, our results demonstrate that IFN-γ can sensitise MB tumour cells to chemo- and radiotherapy through STAT1-dependent and -independent pathways. Using carefully selected animal models with established MB, it remains to be tested whether treatment with IFN-γ might be beneficial for treatment response or dangerous for CNS development.

Conflict of interest statement

None declared.

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